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Medical Management of Renal Lithiasis

Increasing the Protective Urinary Colloids With Hyaluronidase

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SUMMARY

Urine is a highly saturated solution due to the presence of certain colloids. The protective action of urinary colloids is of major importance in preventing precipitation, agglomeration and conglomeration of crystalloids from a super-saturated solution.

If the concentration of such protective colloids is insufficient, stone formation begins or is accelerated. In 680 human subjects, the incidence of stone was found to be almost inversely proportional to the degree of protective urinary colloids present. Urine specimens were subjected to ultramicroscopic examination, determination of electric charge carried by the colloidal particles, determination of the surface tension, and photo-ultramicrographic studies.

Subcutaneous injection of hyaluronidase mixed with physiologic saline solution greatly increases the content of protective colloids in the urine. The colloids are caused to set up to a gel, thereby preventing electrolytes present from crystallizing. They act as excellent dispersing agents and prevent the formation of stone.

Hyaluronidase therapy, using 150 turbidity reducing units every 24 to 72 hours, was effective in preventing calculous formation or reformation during a period of 11 to 14 months in 18 of 20 patients in whom, previously, stones formed rapidly. In a second series of ten patients in whom stones formed rapidly, larger doses of hyaluronidase, averaging 300 turbidity reducing units every 24 to 48 hours, were given. The period of observation at the time of report was from six to ten months. In this group, there was no new stone formation or enlargement of existing stones as evidenced by x-ray studies at 30- to 60-day intervals.

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THE nature and process of kidney stone formation is not completely understood, and the treatment, other than surgical, is still inadequate. The medical management of calculous disease has been primarily directed toward attempts to diminish the concentration of crystalloids excreted in the urine or to free crystalline material from a stone, thus caus-

ing its disintegration. Such therapeutic measures have not proven uniformly successful, with the exception of correcting certain metabolic disorders. Consequently there is great pessimism in the minds of many physicians who are confronted with such problems.

The formation of calculi is a complex process; several concomitant factors are essential in their production. Kidney stones are formed of matter present in two conditions, namely, crystalline and colloidal. They may be defined as concretions composed of urinary crystalloids bound together by and incorporated in a colloidal matrix. Concepts concerning the etiology of stone and consequently its treatment have changed considerably in recent years as the result of intensive laboratory and clinical investigation.[3-8, 10, 11, 13, 16]

The role of protective urinary colloids in the treatment and prevention of stones has, up to quite recently, received little attention. Although Lichtwitz[14] demonstrated the importance of colloids in increasing the solubility of the crystalloidal components in the urine, there is in the medical literature a surprising lack of information concerning further investigation into this problem. Prophylactic and therapeutic efforts have been focused upon preventing the precipitation of colloids and thus indirectly diminishing the formation of urinary deposits.

Colloids or matter in a colloidal state are aggregates of molecules and thus are in a position between the microscopic and molecular dimensions. Their sizes are considered to range from 0.001 Mu to 1 Mu (10^{-7} to 10^{-4} cm.). A colloidal solution, when examined macroscopically or with an ordinary microscope, appears to be clear. It is necessary to view the colloidal particles by reflected light rather than by transmitted light because their dimensions are less than the wave length of visible light. When sols, as these solutions are called, are observed with the ultramicroscope, the colloidal particles appear as bright discs in a state of ceaseless, agitated, zig-zag, erratic, rapid movement known as Brownian molecular motion. The phenomenon constitutes a visual demonstration of molecular kinetic energy. The apparent size of the bright images bears no relationship to the size of the actual particles. It is possible, for practical purposes, to count the number of particles present in the field of the ultramicroscope and thus determine the number in a known volume of fluid (field diameter approximately 0.002 mm.; volume 1 x 10^{-9} ml.).

If the particles of a substance are reduced in size until the dimensions become submicroscopic and are distributed throughout a second medium, they develop characteristic properties attributable to the enormous suface area of the dispersed phase. It has been estimated that one gram of colloidal material in urine has a surface area of approximately 5,000 square meters. One of the most important results of this large surface is the unsaturation of ions located in the surface, causing adsorption of ions dispersed

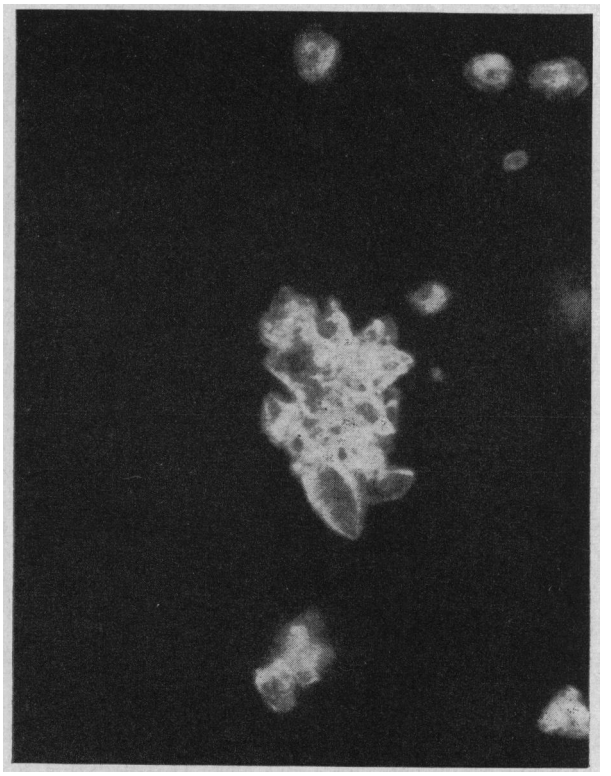


Figure 1.—Ultropak microscopic view × 2,500. Beginning of kidney stone formation by crystallization of urine in white male with rapidly forming bilateral, calyceal stones.

in the surrounding medium and carrying an electric charge of opposite sign. This leads to the formation of electrical charges around the particles, preventing the formation of larger aggregates. Another important factor in preventing precipitation and agglutination of colloids and crystalloids is that sedimentation of finely divided material may be almost entirely counteracted by the Brownian motion of the colloidal particles. Because of the extreme minuteness of colloidal particles, forces come into play which are negligible in greater dimensions. Such forces are dynamic, not static factors.

Urine is a highly saturated solution of extremely complicated composition, in which electrolytes, as well as non-electrolytes, are dissolved in much higher concentration than their solubility in water would indicate. The reason for this is that urine of a healthy person contains colloids which prevent the precipitation of substances in such super-saturated solutions, as long as their degree of dispersity is sufficiently pronounced. It is the protective action of these colloids which is important in preventing precipitation, agglomeration and conglomeration of crystalloids. If the concentration of such protective colloids is insufficient, however, then the crystal nuclei are "sensitized" and stone formation begins or is accelerated (Figure 1).

From what is known of the effect of colloids in increasing the solubility of stone-forming salts and

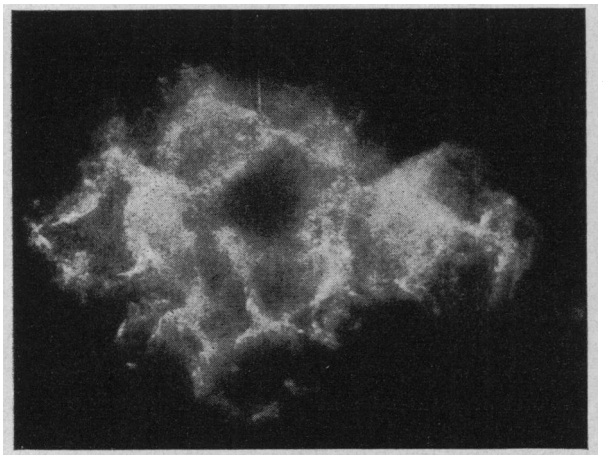


Figure 2.—Ultropak microscopic view, × 2,500. Urine deposit of white male who had constant symptoms of kidney stones. This picture was taken ten minutes after hyaluronidase had been injected. It clearly shows the almost immediate reaction to this medication, because now the solid particles have all become the dispersed part of a jelly-like cluster.

preventing their precipitation, it is logical to assume that if their protective power is diminished or absent, urinary deposits will form more readily. This mechanism probably also applies to other types of calculous formation, whether in the teeth, salivary ducts, pancreas, gallbladder or prostate gland.

During the time that one of the authors (A.J.B.) was stationed on various islands in the Pacific Ocean during World War II, he had an excellent opportunity to study the incidence of stone formation among persons of many different ethnic groups and nationalities. From these observations, and from further investigations in West Florida, which is an area of high stone incidence, it became evident that the only common factor whch applied in the majority of cases was that of the presence or absence of protective urinary colloids in the various cases studied.[3-8] The significance of urinary colloids in the relationship to stone formation was further investigated in a series of 680 subjects.

Specimens of urine were obtained aseptically from both male and female patients and were subjected to ultramicroscopic examination, determination of electric charge carried by the colloidal particles, and determination of the surface tension. The most characteristic specimens were subjected to photo-ultramicrographic studies. For the ultramicroscopic examination, the "colloidal activity" was graded from zero to four; zero representing no colloidal activity; grade one minus, one to two colloidal particles intermittently present in the field; grade one, one to five particles constantly present in the field, grade two, six to ten particles per field; grade three, 11 to 20 particles per field; and grade four, over 20 particles per field.

In the ultramicroscopic studies it was noted that the degree of colloidal activity present in urine samples from which all extraneous sources of colloidal material had been excluded, was almost inversely

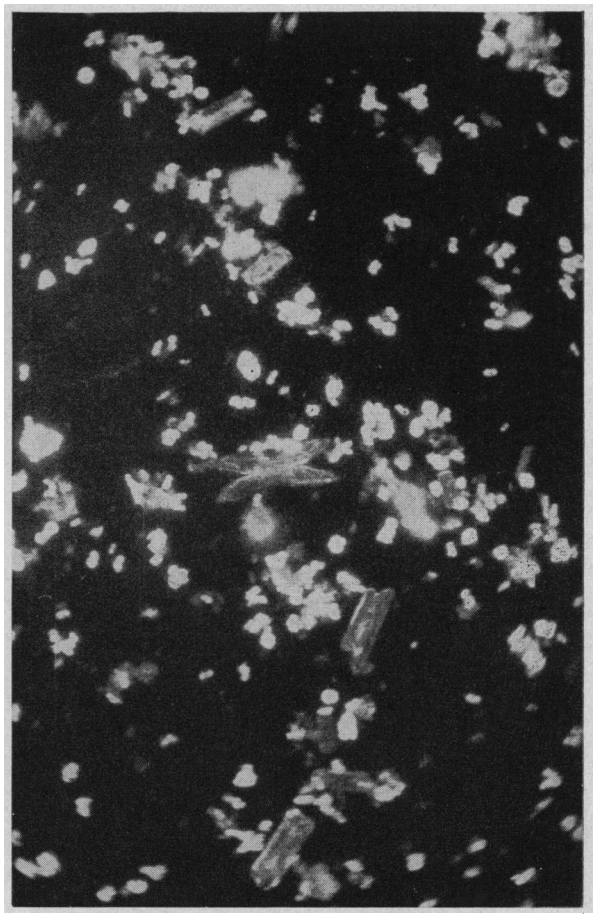


Figure 3.—Ultropak microscopic view, × 2,500. Urine deposit of white male in whom calyceal stones rapidly form bilaterally. Besides a few large crystals, the urine is full of solid crystal micelles which tend to aggregate and eventually grow into larger stones.

proportional to the incidence of stone.[5-8] In the urine of Negroes (who are relatively immune to calculous disease) there was considerably greater colloidal activity than in urine from white subjects. In a series of 250 cases of kidney stones observed during a period of three years, there were only six cases, or 2.4 per cent, in Negroes. Approximately 10 per cent of all patients observed were Negroes. Excretory radiographic studies were routinely made on all private and charity patients.

The urine of females generally had a higher concentration of ultramicroscopically detectable particles than that of males. In practically all statistical series, the incidence of stone is significantly higher in males than in females. In a series of 250 cases of renal lithiasis observed by the authors, there were 167 males and 83 females. The concentration of ultramicroscopically visible colloidal particles in urine from pregnant women greatly surpassed the concentration of such colloidal particles in urine of non-pregnant women. In an analysis of collected series of 49,000 obstetrical cases from various parts of the United States, it was noted that only 15, or 0.03 per cent, were complicated by stone.[2, 9, 12, 15] A par-

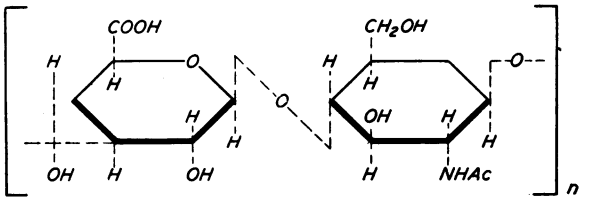
turient woman, with dilatation and stasis of the urinary tract, often complicated by infection, is usually well protected against formation of stone. This would indicate that pregnancy does not predispose to calculous disease, but actually aids materially in preventing stone formation.

Surface tension determinations were made with the pendant drop method[1] from urine of various patients after it had been subjected to ultracentrifuging, whereby all matter visible in the ultramicroscope was removed. The results clearly demonstrated that urine of white females had a considerably higher surface tension than that of Negro females. The surface tension of urine of both white and Negro females decreased during pregnancy. In addition, it was noted that the amount of colloidal particles visible in the ultramicroscope significantly increased as pregnancy progressed.

In electrophoretic studies of the non-centrifuged samples of urine it was observed that urine which had a low surface tension had all been obtained from patients who were free of stone, or those who were relatively immune to stone, such as Negroes. This would indicate quite clearly that this favorable condition is attributable to the presence of capillary-active lyophilic colloids. The presence of these capillary-active agents is the predominant factor in preventing the precipitation and conglomeration of crystalloids and thus preventing the formation of stones. This is due to their acting as excellent dispersing agents and protective colloids which form a reversible gel, which prevents the formation of stone from solid crystals of inorganic matter (Figure 2).

While seeking a protective colloid that is excreted in the urine, it was found that hyaluronidase (Wydase®) not only appreciably reduces surface tension, but, when mixed with physiologic saline solution and injected subcutaneously, also acts to disperse minute particles suspended in urine and will prevent stone formation. This is due to the fact that it causes the natural urine colloids and abundant physiologically increased protective urinary colloids to set up to a gel, thereby preventing the electrolytes present from crystallizing.

Chart 1.—Structural formula of hyaluronic acid.



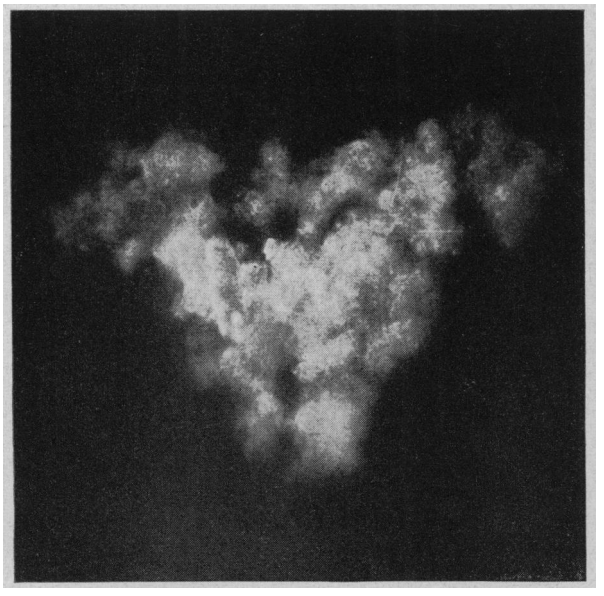


Figure 4.—Ultropak microscopic view, × 2,500. Urine deposit of the same patient as in Figure 3, thirty minutes after hyaluronidase had been injected. To be noted is the complete reaction of this drug, as now the solid particles have all become the dispersed part of a jelly-like cluster and cannot further aggregate and eventually grow into larger stones.

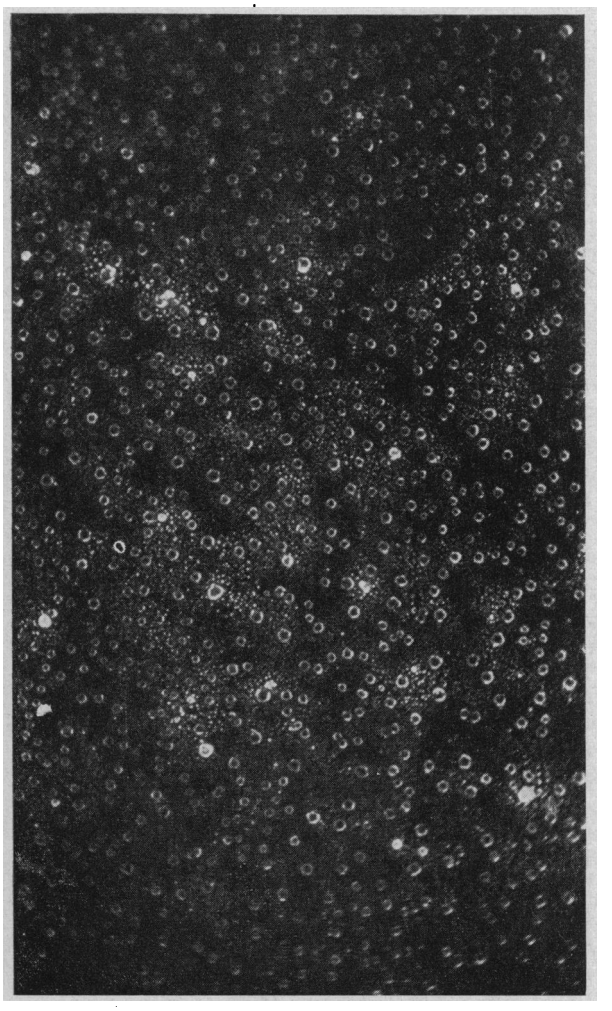


Figure 5.—Ultropak microscopic view, × 2,500. Urine sediment of patient with parathyroid adenoma and multiple, bilateral, renal calculi. The urine is full of solid aggregates of ultramicroscopic-sized unprotected colloidal particles, which tend to conglomerate and eventually grow into larger stones of clinical importance.

Hyaluronidase is an enzyme whose molecular weight has been estimated to be between 60,000 and 70,000. Oxidizing and reducing agents destroy its activity. Hyaluronic acid, a substrate of hyaluronidase, is a linear or spiral polymer whose molecular weight has been estimated at from 200,000 to 2,000,000. It is composed of alternating units of acetyl glucosamine and glucuronic acid (Chart 1). It is a viscous mucopolysaccharide, which in animal tissue seems to bind with water in interstitial spaces, holding cells together in a jelly-like matrix. Hyaluronidase releases hyaluronic acid at the site of injection. The weakened barrier begins to reconstitute itself as the hyaluronidase action is dissipated. During the period of repair, excess hyaluronic acid or a substrate is present in the blood and is excreted in the urine. The effect of hyaluronidase on increasing protective urinary colloids is an indirect one as the hyaluronic acid, or one of its products, when released, is excreted in the urine, thus acting as a protective colloid.

The clinical application of increasing protective colloids by parenteral injection of hyaluronidase is a new concept in the treatment and prevention of renal lithiasis. The first 20 patients subjected to the treatment had multiple, bilateral, and rapidly recurring renal calculi. They had passed numerous stones at regular intervals over a period of years and new stones developed within a period of weeks or a few months. In these patients, no regimen of therapy had been effective in reducing the formation or reformation of stone before hyaluronidase therapy was instituted. Subcutaneous injection of 150 turbidity reducing units of hyaluronidase mixed with 1 cc. of saline was regulated individually by observing the duration of increased colloidal activity after injection of the drug, so that this protective activity was maintained at an increased level. This varied from 19 to 120 hours and the average was from 24 to 72 hours. Each patient was investigated with regard to all known etiological factors as relates to stone formation. At the time of this report these patients had been receiving hyaluronidase therapy for from 11 to 14 months and no other form of therapy to combat stone formation had been employed during that time. In 18 of these 20 patients (90 per cent), no new stone formation or increase in size of existing stones occurred when the drug was taken properly, as is evidenced by roentgenograms taken at 30-day to 60-day intervals. A second series of ten patients, in all of whom stones formed rapidly, received larger doses of hyaluronidase, averaging 300 turbidity reducing units every 24 to 48 hours. At the time of this report, after periods of treatment ranging from six to ten months, there was no new stone formation or increase in size of existing stones, as evidenced by x-ray films taken at 30- to 60-day intervals. Three cases are summarized and only significant findings are noted.

REPORT OF THREE CASES

Case 1. A 34-year-old male had had symptoms for seven years. Stones composed of calcium phosphate and ammonium magnesium phosphate were passed at intervals of one to five months. The degree of protective urinary colloidal activity was extremely low (Figure 3). In roentgenograms, innumerable, bilateral calyceal stones were noted. Hyaluronidase therapy was started October 2, 1950. X-ray films were taken at intervals of one to two months and there was no evidence of new stones or growth of existing stones during the 11 months after therapy was begun. Protective urinary colloids were maintained at an elevated level (Figure 4). The patient passed stones on two occasions when therapy was discontinued for three weeks each time. Otherwise, he was asymptomatic.

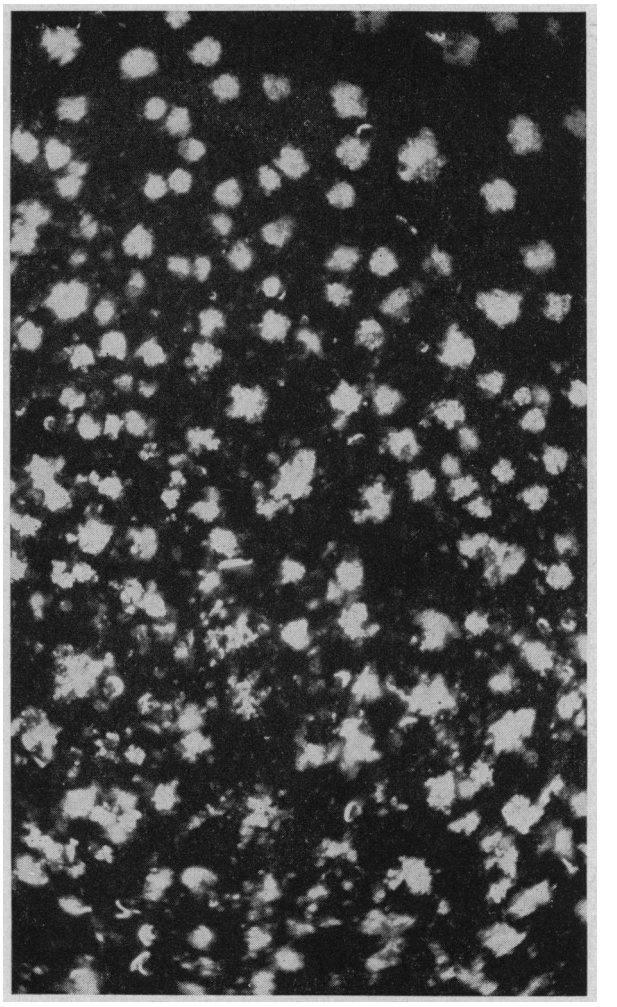


Figure 6.—Ultropak microscopic view, × 2,500. Deposit in urine of same patient as in Figure 5 thirty minutes after injection of hyaluronidase. Now forming in the urine are only highly solvated gel clusters which are readily redispersed when diluted.

Case 2. A woman, 50 years of age, who had had symptoms for 25 years, passed stones composed of calcium phosphate at intervals of one to three months. The protective urinary colloid content was extremely low (Figure 5). In roentgenograms, innumerable bilateral calyceal stones were observed. The serum calcium content was 14.2 mg. per 100 cc. and the phosphorus content was 1.9 mg. per 100 cc. In a 24-hour urine specimen, the calcium content was 1.1 gm. per 100 cc. Hyaluronidase therapy was started December 12, 1950. X-ray films were taken at three- to four-week intervals and no new stones or growth of existing stones

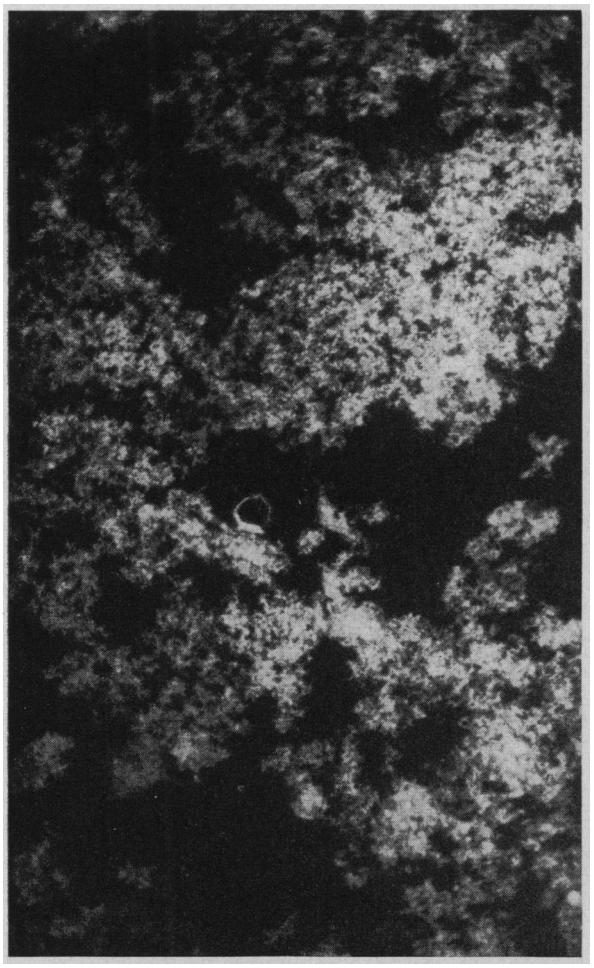


Figure 7.—Ultropak microscopic view, × 2,500. Urine sediment from a white female with bilateral, renal calculi, and a severe urinary tract infection. The urine is full of aggregates of colloidal particles (non-protective colloid composed of degradation products of leukocytes, erythrocytes, bacteria and exudates) and one crystal near the center of the plate.

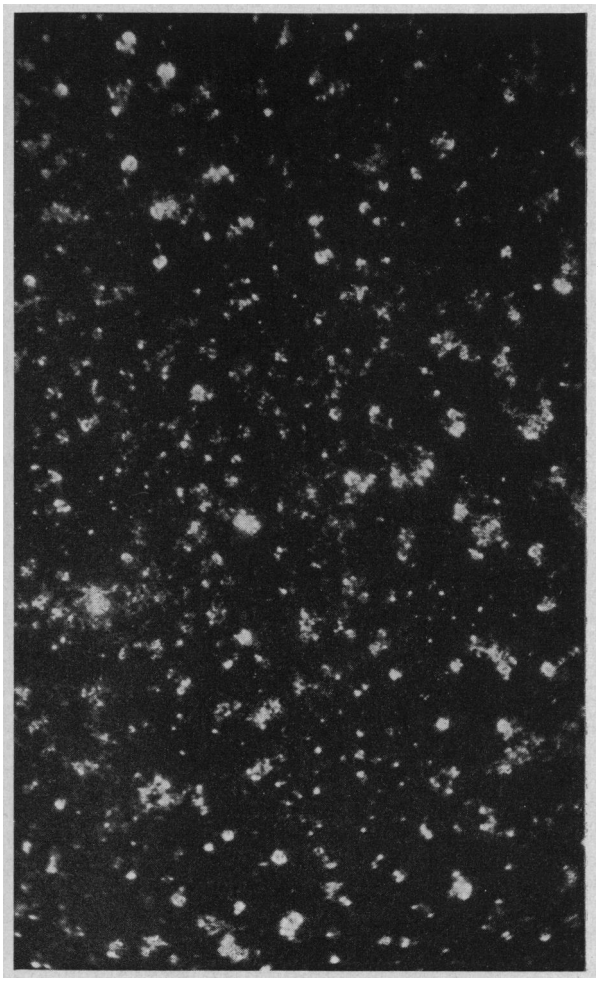


Figure 8.—Ultropak microscopic view, × 2,500. Urine of same patient as in Figure 7 thirty minutes after injection of hyaluronidase. Now forming in the urine are only highly solvated gel clusters which prevent further agglomeration and conglomeration of crystalloids.

was noted after therapy was begun. Urinary protective colloids were maintained at an elevated level (Figure 6). The patient passed no stones and had no symptoms after treatment was started.

CASE 3. A woman 36 years of age who had had symptoms for five years passed stones composed of calcium phosphate and ammonium magnesium phosphate every two to three months. The content of protective colloids in the urine was low (Figure 7). Bacillus coli grew on a culture of the urine. A cluster of small stones in a dilated lower calyx on the left side and multiple small calyceal stones in the right kidney were observed in roentgenograms. Hyaluronidase therapy was started and protective urinary colloids were maintained at an elevated level (Figure 8). X-ray films were taken every one to two months and there were no new stones or enlargement of existing stones after nine months. There were no symptoms after hyaluronidase therapy was started.

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